VALIDATION AND COMPARISON; A STEP TOWARDS THE AUTOMATION FOR MEASURING THE ERYTHROCYTE SEDIMENTATION RATE

Sumera Shaikh[1]*, Fatima Meraj[1], Mamona Mushtaq[2], Sumana Shahid[1] and Saba Jamal[3]

[1]*Department of Hematology, The Indus Hospital and Health Network, Karachi-751900, Pakistan*
[2]*Indus Hospital Research Center, The Indus Hospital and Health Network, Karachi-751900, Pakistan*
[3]*The Indus Hospital Blood Center, The Indus Hospital and Health Network, Karachi-751900, Pakistan*

Email addresses:
SS: sumaira.shaikh@tih.org.pk; FM: fatima.meraj@tih.org.pk
MM: mamona.mushtaq@tih.org.pk; SS: sumana.shahid@tih.org.pk
SJ: saba.jamal@tih.org.pk

ABSTRACT

The erythrocyte sedimentation rate (ESR) is widely used indicator to monitor the activity of various inflammatory diseases. Ves-Matic Cube 30 is an automated instrument based on the modified Westergren principle, used to measure the ESR. This study is aimed to assess the analytical performance of the Ves-Matic analyzer as per the recommendation of the International Council for Standardization in Haematology (ICSH) in comparison to the standard method. Herein the method validation was performed which included the determination of intra-run, inter-run precision and reference range verification. Further, the automated method was compared to the reference method by plotting the Passing-Bablok regression equation and the agreement assessment using the Bland and Altman test. Intra-run precision assessed with patient's samples at three levels yielded the coefficients of variation (CVs) of 15.06%, 7.62% and 3.16% whereas, inter-run CVs of 12.29% and 5.68% for the quality control samples with normal and abnormally high ESR range, respectively. A strong positive correlation was observed between Westergren and Ves-Matic methods with Spearman's coefficient of 0.97 (p value of < 0.001). The Passing-Bablok regression analysis yielded an intercept and slope of -0.904 and 0.957, respectively. The Bland and Altman analysis revealed good agreement with a bias of 2.1 mm/hour between the tested analytical methods. Our results obtained indicated that the Ves-Matic Cube 30 analyzer can be used in high workload clinical settings for ESR measurement as the generated results were in concordance with the reference method.

Key Words: Erythrocyte sedimentation rate, Ves-Matic Cube 30, Bland and Altman, Passing-Bablok regression.

INTRODUCTION

The erythrocyte sedimentation rate (ESR) is one of the most widely used hematological tests in clinical settings. This technique was first introduced by Dr. Edmund Biernacki in 1894, followed by an independent description by Drs. Hirszfeld, Fahraeus, and Westergren (Kratz *et al.,* 2017). In general, ESR is the estimation of the length to which red blood cells (RBCs) settle down as sediment in a predefined time frame. The principle of working of ESR includes an intricate physicochemical phenomenon encompassing distinctive phases which include the RBCs aggregation followed by their assemblage as rouleaux formation, sedimentation, and erythrocyte packaging (Lapic *et al.,* 2019). The sedimentation rate of RBCs is dependent on the levels of acute-phase proteins in the blood circulation, predominantly fibrinogen. During circulation acute phase proteins modulate the dielectric constant in the blood, counteracting the negative charges on the surface of RBCs causing repulsion between them consequently, opposing aggregation. The increase in ESR thus serves as an indicator of inflammation (Alende-Castro *et al.,* 2019).

Since numerous other pathophysiological and physiological conditions may modulate this phenomenon thus rendering ESR as a non-specific marker of inflammation (Lapic *et al.,* 2019). Despite being non-specific, ESR remains a frequently prescribed test and its medical utility remains unchanged. The measurement of ESR can be informative in assisting the diagnosis and monitoring the activity of inflammatory diseases for instance giant cell arteritis, rheumatoid arthritis, or infections *etc.* Correspondingly, the ESR may also serve as a useful indicator and predictor of lupus activity and organ damage respectively (Curvers *et al.,* 2010; Lapic *et al.,* 2020a).

Several methods have been devised for measuring the ESR e.g. Westergren method, Zeta sedimentation ratio, Wintrobe's method, and micro-ESR (Narang *et al.,* 2020). However, the Westergren method is regarded as a "gold standard" method and is endorsed by the International Council for Standardization in Haematology (ICSH) as a reference method (Kratz *et al.,* 2017). This method is simple, cheap, and can easily be done. However, there are various drawbacks including the risk of contamination, significant volume of blood required, and comparatively

***Corresponding author:**
Dr. Sumera Shaikh (M.B.B.S, F.C.P.S; Consultant Hematologist, Department of Hematology, The Indus Hospital and Health Network, Karachi-751900, Pakistan); Phone number: 03323641967

increased duration for analysis (>1 hour) (Erdogan *et al.,* 2021; Orkmez *et al.,* 2021). Consequently, during the last two decades novel automated and semi-automated methods have been introduced to overcome the limitations of the conventional Westergren method. These methods are much better suited in terms of reproducibility, the safety, reduced processing time, and lessen biohazards risks (Guarner *et al.,* 2021).

These new procedures are either regarded as modified Westergren methods or alternate methods based on the extent of diversion in comparison to the standard method. Moreover, it is also acclaimed that novel technologies and analyzers have to be thoroughly evaluated prior to their utility in routine practice. The procedures efficient enough to yield comparable results to the Westergren method with diluted blood in 1 h or normalized to 1 h are of clinical significance (Kratz *et al.,* 2017; Jou *et al.,* 2011).

The present study aims to evaluate the performance of the Ves-Matic Cube 30 analyzer (Disease Diagnostica Senese, Siena, Italy) and to compare its performance in reference to the Westergren method.

MATERIALS AND METHODS

A comparative study of analytical methods was conducted in the department of hematology at The Indus hospital and Health Network Karachi, Pakistan after getting ethical approval from the hospital's ethical review committee (IHHN_IRB_2021_04_015). The investigation is mainly divided into two phases, method validation, and method comparison.

Method Validation

1. Intra-run Precision

Three patient samples representing low (0-20mm/ hour), middle (21-80 mm/h) and high (51- >100mm/h) ESR were analyzed in 10 replicates each.

2. Inter-run Precision

Inter-run precision was obtained from analysis of commercial control samples on two levels (normal and abnormally high) in triplicate for seven consecutive days.

3. Reference Interval Validation

Whole blood samples from 10 healthy males and 10 healthy females were used to test whether the analyzer is efficient enough in providing an output for the samples within the reference range.

The mean, standard deviation, and coefficient of variation were calculated, inter-precision was also accessed by Levey and Jennings plot (Levey and Jennings, 1950).

Method Comparison

The method comparison study was conducted on 30 samples (highly lipemic and hemolyzed samples were excluded from the study) and readings were obtained from the manual as well as automated method. The comparison between the two methods was assessed by plotting agreement between the two, utilizing the Bland and Altman test (Bland and Altman, 1986; Gerke, 2020). Moreover, linear regression was also plotted utilizing the Passing and Bablok's (Passing and Bablok, 1983) regression equation. The agreement and regression tests were performed via XLSTAT24 (Addinsoft, 2019).

Principle of Working

1. Manual Westergren Method

The whole blood samples (1.6 mL) were drawn by venipuncture and placed in vacuum blacktop tubes containing Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The content was homogenized by manual tapping. The blood in the tubes was aspirated into pipettes (diameter: 4.5x230 mm) followed by the subsequent placement in a vertical upright manner for one hour at room temperature. The ESR was recorded via visual inspection as the length traveled by RBCs from the top of the pipette to the upper limit of RBCs sediment (Dewi *et al.,* 2019).

2. Ves-Matic Cube 30

The Ves-Matic Cube 30, an automatic bench analyzer allows the determination of the ESR of 30 samples at a time on the hematocrit tube. The Ves-Matic Cube 30 analyzer is based on the modified Westergren method. The process starts with the automatic homogenization of the blood for two minutes with EDTA to permit erythrocytes

disaggregation. It is followed by the placement of the samples onto the test tube holder chain. An optoelectronic light source initially scans the tubes and measures the initial height of the blood column. The samples are then incubated for 20 minutes and afterward the shift in optical density is recorded which is the difference between the optical density from plasma layer to sediment layer. The obtained readings were subjected to automatic conversion in standard Westergren units as per the manufacturer's recommendations (Bogdaycioglu *et al.*, 2015; Cerutti *et al.*, 2011).

RESULTS

Method Validation

The results of inter-run and intra-run precision utilizing the quality control and patient samples are presented in Table 1. Analysis of commercial control samples for 7 consecutive days in triplicate yielded an inter-run CV of 12.29% for the normal range and 5.68% for the abnormal range. Obtained intra-run CVs for commercial samples were 9, 8, 0, 9, 8, 12, 7 and 0.8 1.6, 1.6, 2.4, 10.4, 5.1, and 5.4% for normal and high-level quality control samples, respectively.

Table 1. Intra-run precision achieved by analyzing three levels of patient samples (high, low and normal) in 10 replicates.

Samples	Mean ESR (mm) (n=10)	SD (±)	CV (%)
Low (0-20mm/ h)	2.10	0.32	15.06
Middle (21-80 mm/h)	25.10	1.91	7.62
High (51- >100mm/h)	104.60	3.31	3.16

SD: standard deviation, CV: coefficient of variation

The Levey Jennings plot for inter-run precision is depicted in Fig. 1 which also indicated that most of the data points are within acceptable deviation range. Furthermore, the reference ranges were also calculated which indicated ESR within the range of 4-25 mm/hour for females and 2-9 mm/hour for males and mean ESR of 12.70 ± 7.54 and 4.80 ± 1.93 for females and males, respectively.

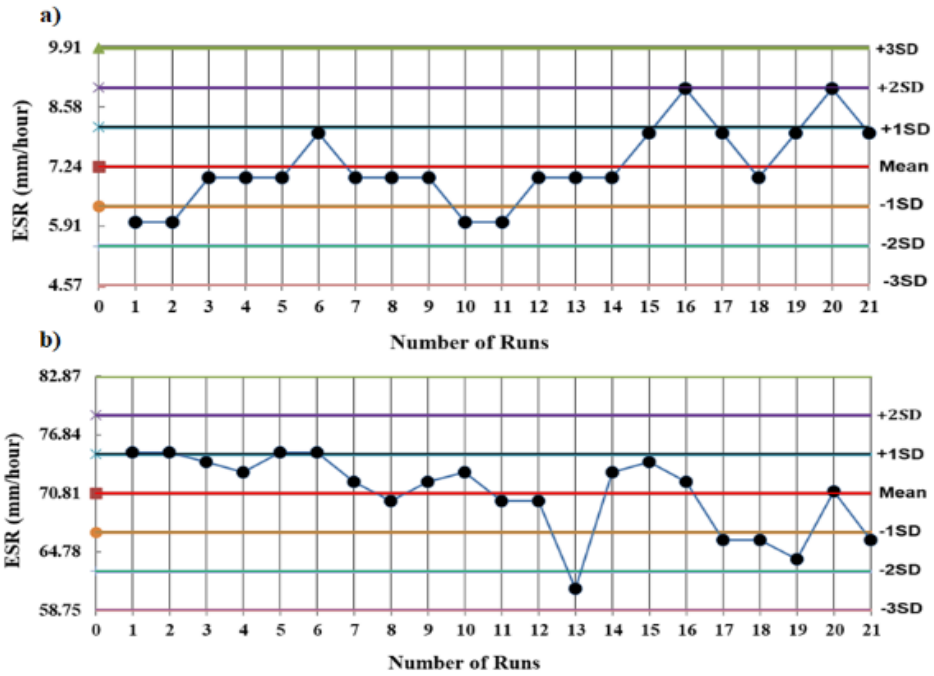

Fig.1. Levey-Jennings Plot for quality control samples a) with normal range b) abnormally high range.

Method Comparison

The study population for analytical techniques comparison consisted of 30 patient samples and exhibited mean ESR of 8.40 ± 5.23, 29.00 ± 6.42, 66.44 ± 19.53 and 5.60 ± 2.76, 29.00 ± 5.33, 62.55 ± 16.58 mm/h for normal, middle, and higher range of ESR calculated by automated and manual methods respectively. The strong positive correlation (95%, CI: 0.93-0.99, p <0.001) was obtained from Spearman's correlation test (Table 2).

Table 2. Analysis of the Spearman's Correlation test on the erythrocyte sedimentation rate (ESR) measurement methods.

Samples	n	Method	Correlation Coefficient (r)
Spearman's Test	30	Manual	0.97
	30	Ves-Matic Cube 30	

Figure 2 depicts a regression analysis used to evaluate the validity of the automated method in compared to the standard method.

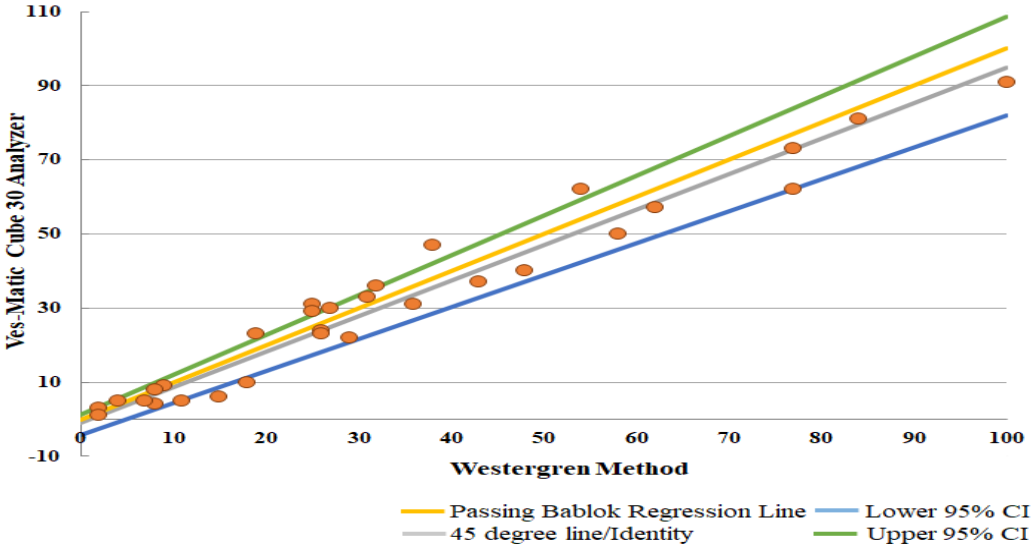

Fig. 2. Passing and Bablok plot displaying the regression curve between the reference method and Ves-Matic Cube 30 analyzer.

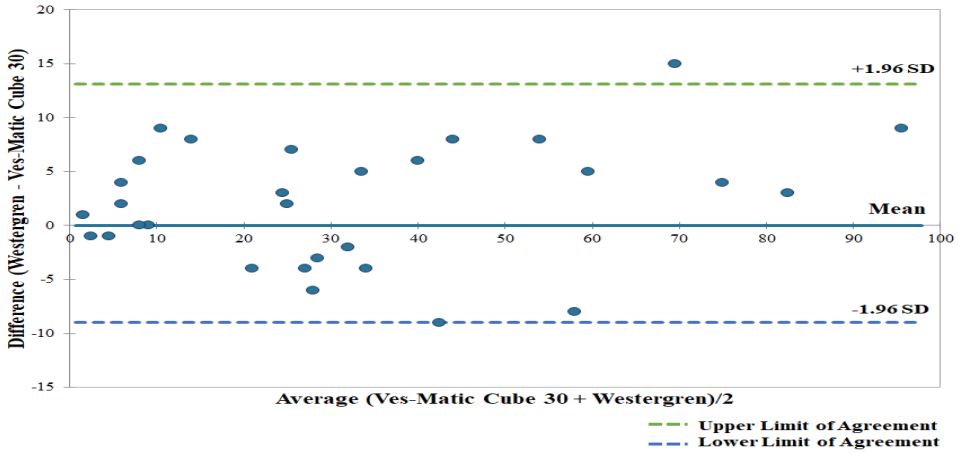

Fig. 3. Bland and Altman plot displaying the agreement between the Westergren and Ves-Matic Cube 30 method.

The regression equation was y = -0.904 + 0.957 x. Bland and Altman's plot defined a bias (mean difference) of 2.1 and a standard deviation of 5.63. While 95% limit of agreement range from -8.944 to 13.144 units. Fig. 3 represents the agreement plot between the tested methods.

DISCUSSION

The ESR is a simple test and perhaps the most extensively measured index of acute inflammatory responses in clinical laboratories. Though lacks specificity, ESR is still in frequent use by clinicians in diseases diagnosis consequently guiding treatment options and prognostication (Chauffaille *et al.,* 2021; Tishkowski and Gupta, 2021).

The conventional Westergren method for the estimation of ESR has served the medical community not less than 70 years however, the Westergren method being manual, possess some inherent limitations. The wide range of variables and the multiple tasks involved can lead to erroneous results whereas the obnoxious risk of infection to the laboratory practitioners and relatively long analysis time cannot be disregarded (Erdogan *et al.,* 2021; Happe *et al.,* 2002)

Consequently, newer automated and semi-automated methods have been introduced that are much better suited in terms of safety and time. Furthermore, the recently devised ESR analyzers bypass the additional dilution steps and thus enhance practitioner's safety and optimize laboratory workflow (Niyibizi *et al.,* 2018). The Ves-Matic is one of such modern automated analyzers used for the estimation of ESR (Pieri *et al.,* 2021; Sezer *et al.,* 2013). However, prior to the implementation of such automated techniques in routine laboratory practices, method validation is of central importance. In this regard, the present investigation was undertaken to validate and compare the Ves-Matic analyzer with the conventional Westergren method. Such validation studies enable the laboratory technologists to use methods that are more appropriate and convenient in routine settings replacing the time taking and laborious procedures to ensure comparability with the standard method.

This study indicated that Ves-Matic Cube 30 presents with satisfactory precision characteristics as recommended by International Council for Standardization in Haematology (ICSH). A much better intra-run precision of Ves-Matic Cube 30 was revealed as indicated by the least standard deviation and hence lower coefficients of variation than a previously reported for iSED (Lepic *et al.,* 2020b). The least dispersion around the mean was observed for ESR under the normal range followed by the middle range. Likewise, when subjected to intra-run precision utilizing commercial control samples at both estimated levels yielded low CVs with decreased precision for normal range in comparison to high range samples. Our results are in accordance with a number of other studies reported in past aimed at the validation regardless of the measurement tool utilized or the very different underlying principle (Erdogan *et al.,* 2021; Dewi *et al.,* 2019; Cerutti *et al.,* 2011). Owing to the reasonable precision, it was deduced that the tested automated analyzer is efficient enough to be subjected for comparability testing against the internationally recognized method. The comparison between the Ves-Matic Cube 30 and Westergren method revealed a higher positive correlation, signifying the existence of linear relation and no significant differences between the two methods.

The two of the tested analytical methods for ESR estimation were further compared by generating a Passing Bablok regression plot. It determines bias and is considered a model test for comparing clinical methods due to its robustness against outliers and allows imprecision both in the X and Y variables. Passing–Bablok regression analysis comparing ESR measurements from Ves-Matic Cube 30 relative to the measurements obtained from the manual method indicated reliable estimation by the automated method as most of the data points were within the 95% confidence interval.

The 95% limits of agreement were also calculated between the manual and automated methods utilizing the Bland and Altman analysis. We inferred that for the evaluated ESR values measured by Ves-Matic Cube 30 for 95% of the subjects would be 8.94 mm/h below the manual ESR or 13.14 mm/hour above it. The least dispersion and maximum correlation signify that the ESR measurement by two methods showed good agreement.

In our study, more variations were observed for low followed by high ESR values, between the two methods. For low ESR values, the calculated mean difference was 2.8 ± 3.73 (95% CI: 0.09 to 0.94; with least correlation coefficient 0.729; $p < 0.017$), for middle there was no difference recorded at average (95% CI: 0.09 to 0.93; correlation coefficient 0.696; $p < 0.017$) and for high ESR values, mean difference was 3.899 ± 7-849 (95% CI: –0.55 to 0.99 with correlation coefficient 0.918; $p < 0.001$).

However, it is also evident from the results that regardless of efforts made in the past years in the pursuit of standardization of the ESR measuring method, the variation can still be observed. It can be attributed to the differential ability of the tested methods involving the measurement of different phases of the erythrocyte's sedimentation process.

Conclusion

The tested method (Ves-Matic Cube 30) showed a good correlation to the Westergren method (as indicated by Spearman's correlation test, regression, and agreement analysis). These findings indicate that Ves-Matic Cube 30 might be a reliable and suitable alternate to Westergren in a high workload clinical setting. This study was conducted on a small sample size, however, provides a basis for further testing. Hence it is suggested to further test the validity of the Ves-Matic Cube 30 analyzer.

DECLARATIONS

Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Grant Support & Financial Disclosures

The authors did not receive support from any organization for the submitted work.

Authors Contribution

SS* is the corresponding author and contributed to study idea, design and manuscript review. SS performed the experiment under the supervision of SS* and FM. MM did the data analysis and manuscript write-up. FM and SJ did a critical review of the manuscript.

Patients consent

The present investigation was conducted on retrospective data, therefore, it was not possible to take consent from the patients. Though, data confidentiality was maintained throughout the study and prior to the study the approval was acquired from the hospital's review board (IHHN-IRB_2021_04_015).

REFERENCES

Addinsoft, A. (2019). *XLSTAT statistical and data analysis solution*. Long Island, NY, USA.

Alende-Castro, V., M. Alonso-Sampedro, N. Vazquez-Temprano, C. Tuñez, D. Rey, C. García-Iglesias, B. Sopeña, F. Gude and A. Gonzalez-Quintela (2019). Factors influencing erythrocyte sedimentation rate in adults: new evidence for an old test. *Medicine*, 98(34): e16816. DOI: 10.1097/MD.0000000000016816.

Bland, J.M. and D. Altman (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet,* 327(8476): 307-310. DOI: 10.1016/S0140-6736(86)90837-8.

Boğdaycioğlu, N., F.M. Yilmaz, S. Sezer and E. Oğuz (2015). Comparison of iSED and Ves-Matic Cube 200 erythrocyte sedimentation rate measurements with Westergren method. *Journal of Clinical Laboratory Analysis*, 29(5): 397-404. DOI: 10.1002/jcla.21786.

Cerutti, H., C. Muzzi, R. Leoncini, C. Scapellato, A. Cortelazzo, E. Furlani and R. Guerranti (2011). Erythrocyte sedimentation rate measurement by VES Matic Cube 80 in relation to inflammation plasma proteins. *Journal of Clinical Laboratory Analysis*, 25(3): 198-202. DOI: 10.1002/jcla.20457.

Chauffaille, de M.L., I.Y. Takihi, W.H. Prieto, P.D. Russo, A.F. Sandes, A.B. Perazzio, M.C. Silva and M.V. Gonçalves (2021). New reference values for the old erythrocyte sedimentation rate. *International Journal of Laboratory Hematology*, 43(4):O214-O217. DOI: 10.1111/ijlh.13523.

Curvers, J., J. Kooren, M. Laan, E. van Lierop, D. Van de Kerkhof, V. Scharnhorst and M. Herruer (2010). Evaluation of the Ves-Matic Cube 200 erythrocyte sedimentation method: comparison with Westergren-based methods. *American Journal of Clinical Pathology,* 134(4): 653-660. DOI: 10.1309/AJCPMEWW62BGQHJH.

Dewi, M.M., S. Herawati, N.K. Mulyantari and I. P. Prabawa (2019). The comparison of erythrocyte sedimentation rate (ESR) modify Westergren Caretium Xc-A30 and Westergren Manual in Clinical Pathology Laboratory, Sanglah General Hospital, Denpasar, Bali. *Bali Medical Journal*, 8(2): 396-399. DOI: 10.15562/bmj.v8i2.1401.

Erdogan, S., R. Firat, G. Avcioglu, G. Yilmaz, O. Erel and F.M. Yilmaz (2021). Is Vision C interchangeable with the modified Westergren method for the erythrocyte sedimentation rate? *Turkish Journal of Biochemistry,* DOI: 10.1515/tjb-2020-0090.

Gerke, O. (2020). Reporting standards for a Bland–Altman agreement analysis: A review of methodological reviews. *Diagnostics,* 10(5): 334. DOI: 10.3390/diagnostics10050334.

Guarner, J., H.K. Dolan and L. Cole (2015). Erythrocyte sedimentation rate: journey verifying a new method for an imperfect test. *American Journal of Clinical Pathology,* 144: 536-538. DOI: 10.1309/AJCPQO81BGTKTVJJ.

Happe, M.R., D.F. Battafarano, D.P. Dooley, T.A. Rennie, F.T. Murphy, T.J. Casey and J.A. Ward (2002). Validation of the Diesse Mini-Ves erythrocyte sedimentation rate (ESR) analyzer using the Westergren ESR

method in patients with systemic inflammatory conditions. *American Journal of Clinical Pathology*, 118(1): 14-17. DOI: 10.1309/YHYL-8XTH-5JM2-43EJ.

Jou, J.M., S.M. Lewis, C. Briggs, S.H. Lee, B. De La Salle, S. McFadden and International Council for Standardization in Haematology (ICSH) (2011). ICSH review of the measurement of the erythocyte sedimentation rate. *International Journal of Laboratory Hematology,* 33(2): 125-132. DOI: 10.1111/j.1751-553X.2011.01302.x.

Kratz, A., M. Plebani, M. Peng, Y.K. Lee, R. McCafferty, S.J. Machin and International Council for Standardization in Haematology (ICSH) (2017). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. *International Journal of Laboratory Hematology,* 39(5): 448-457. DOI: 10.1111/ijlh.12693.

Lapić, I., A. Padoan, D. Bozzato and M. Plebani (2020a). Erythrocyte sedimentation rate and C-reactive protein in acute inflammation: meta-analysis of diagnostic accuracy studies. *American Journal of Clinical Pathology*, 153(1): 14-29. DOI: 10.1093/ajcp/aqz142.

Lapić, I., E. Piva, F. Spolaore, G. Musso, F. Tosato, M. Pelloso and M. Plebani, (2019). Ves-Matic CUBE 200: is modified Westergren method for erythrocyte sedimentation rate a valid alternative to the gold standard? *Journal of Clinical Pathology*, 72(10): 716-719. DOI: 10.1136/jclinpath-2019-205873.

Lapić, I., M. Miloš, F. Tosato, E. Piva, R. Zadro, D. Rogić and M. Plebani (2020b). Analytical validation of the iSED automated analyzer for erythrocyte sedimentation rate. *International Journal of Laboratory Hematology*, 42(2): 109-115. DOI: 10.1111/ijlh.13120.

Levey, S. and E. Jennings (1950). The use of control charts in the clinical laboratory. *American Journal of Clinical Pathology*, 20(11_ts): 1059-1066. DOI: 10.1093/ajcp/20.11_ts.1059.

Narang, V., S. Grover, A.K. Kang, A. Garg and N. Sood (2020). Comparative Analysis of Erythrocyte Sedimentation Rate Measured by Automated and Manual Methods in Anaemic Patients. *Journal of Laboratory Physicians*, 12(4): 239. DOI: 10.1055/s-0040-1721155.

Niyibizi, J.B., R.K. Miruho and P. Onyambu (2018). Correlating Automated IRIA ESR Analyzer and Westergreen Method for Determination of Erythrocyte Sedimentation Rate. *Asian Hematology Research Journal*, 1 (4): 1-6. DOI: 10.9734/AHRJ/2018/45295.

Orkmez, M., S. Orhan, M.A. Bozdayi and M. Tarakcioglu (2021). Comparison of the StaRRsed Interliner device with Westergren method in erythrocyte sedimentation rate measurement. *International Journal of Laboratory Hematololgy*, 43(4):616-622. DOI: 10.1111/ijlh.13444.

Passing, H. W. and Bablok (1983). A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. *Journal of Clinical Chemistry and Clinical Biochemistry*, 21: 709-720. DOI: 10.1515/cclm.1983.21.11.709.

Pieri, M., S. Pignalosa, M.A. Perrone, C. Russo, G. Noce, A. Perrone, A. Terrinoni, R. Massoud and S. Bernardini (2021). Evaluation of the Diesse Cube 30 touch erythrocyte sedimentation method in comparison with Alifax test 1 and the manual Westergren gold standard method. *Scandinavian Journal of Clinical and Laboratory Investigation*, 81: 181-186. DOI: 10.1080/00365513.2021.1881996.

Sezer, S., F.M. Yilmaz, O. Kaya *et al*. (2013). Evaluation of Ves-Matic Cube 200 for erythrocyte sedimentation rate determination. *Journal of Clinical Laboratory Analysis*, 27(5): 367-372. DOI: 10.1002/jcla.21612.

Tishkowski, K. and V. Gupta (2021). *Erythrocyte Sedimentation Rate*. Treasure Island (FL): StatPearls Publishing.

(*Accepted for publication December 2021*)